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Expression of mouse IgA by transgenic mice, pigs and sheep*

The development of transgenic animal technology allows the introduction of desired traits into the germ line of mice and other animals. Since the production of antibody to various polysaccharide antigens can be protective against pathogenic bacteria, we generated transgenic mice, sheep and pigs carrying genes encoding the mouse α and κ chains for antibodies against phosphorylcholine (PC) to determine whether transgene antibody might be used to influence susceptibility to disease. High serum levels of mouse IgA were detected in transgenic mice and pigs but not in transgenic sheep. It has been noted that transgene immunoglobulin expression can suppress endogenous immunoglobulin gene rearrangement and expression, and indeed, in one mouse line, expression of the transgene resulted in < 10% of spleen B cells expressing endogenous IgM. Despite this suppression, significant levels of endogenous IgM were still secreted into the serum. Suppression of endogenous IgM expression was not seen in other mouse lines, nor was it seen in transgenic pigs. In the transgenic pigs, the mouse IgA was detected in the serum despite the absence of an intact mouse κ transgene, so the secreted antibody presumably included pig light chains. Little if any of the mouse IgA in these sera showed binding specificity for PC. In one of the founder sheep, mouse IgA was detectable in peripheral lymphocytes but not in serum. Mouse κ expression was not detected in the transgenic sheep harboring an intact κ transgene. These results illustrate the potential of introducing beneficial traits such as germ-line-encoded immunity into large mammalian species.

1 Introduction

The development of transgenic animal technology has allowed valuable new experimental approaches to the study of *in vivo* processes. While most recent studies have been made in transgenic mice, generation of transgenic large mammals extends the potential utility of the system [1, 2]. For example, studies on medical physiology often require the use of larger animals, partly because rodents are too small for measurements of small changes in physiological parameters, and partly because rodent physiology is different from that of humans. For similar reasons, the potential for gene therapy must also be tested in large animal experimental models. Yet the potential of the transgenic animal technology is not limited to basic biological questions, for the technology can be used to develop both commercial and clinical applications. Thus, transgenic large animals may be used as a source for large amounts of therapeutic proteins such as human hormones, hemoglobin or clotting factors.

One potential application of the generation of transgenic large animals is the introduction of beneficial genes to improve health and disease resistance. Expression of a transgenic Ig specific for a common pathogen could provide an animal with congenital immunity for that pathogen. In studies on Ig transgenic mice however, the expression of the Ig transgene sometimes resulted in significant inhibition of gene rearrangement and expression of endogenous Ig genes [3–7]. It was therefore of great concern whether the Ig transgene approach would in fact cripple the immune system rather than augment it. We have begun a series of studies to address these issues, and we present here our initial findings of transgenic mice, pigs and sheep expressing a transgene mouse Ig. Our results indicate that mouse Ig transgenes are expressed in a tissue-specific manner in large mammalian species, and that their expression need not interfere with expression of endogenous Ig.

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2 Materials and methods

2.1 Generation of transgenic animals

Genes encoding the mouse α and κ chains from antibodies against PC were prepared and microinjected into fertilized eggs from mice, pigs and sheep as described previously [1]. The mouse α heavy chain gene cloned from S107 was generously provided by Drs. P. Gregor, S. Brown and S. Morrison. The mouse κ light chain gene cloned from MOPC167 has been used previously to generate transgenic mice [5] and was generously provided by Dr. U. Storb. The same preparation of DNA was used for all three animal species.

2.2 Staining of cells and histological sections

For FCM staining of mouse cells, biotinylated anti-mouse IgA (Zymed, San Francisco, CA), anti-κ, PE-streptavidin

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(both Southern Biotechnology, Birmingham, AL), and FITC anti-mouse IgM (Vector, Burlingame, CA) were used on freshly isolated spleen cells, which were subsequently fixed in 1% paraformaldehyde. For cytospin and cryostat slides, cells were stained for mouse and pig Ig using biotinylated anti-mouse IgA, and biotinylated anti-pig IgG (Southern Biotechnology), followed by streptavidin-horseradish peroxidase (Jackson Immunoresearch, Avonsdale, PA), and detection using diaminobenzidine as a chromagen. Slides were counterstained with hematoxylin.

2.3 Assays of serum Ig

Quantitation of serum Ig was done using radial immunodiffusion plates specific for mouse IgA, mouse IgM and pig IgG (The Binding Site). ELISA quantitation of titers against PC were done using a sandwich technique consisting of PC-BSA-coated microtiter dishes. Bound Ig was detected using horseradish peroxidase-conjugated goat anti-mouse antibodies (Kirkegaard and Perry Labs., Gaithersburg, MD) followed by addition of the substrate

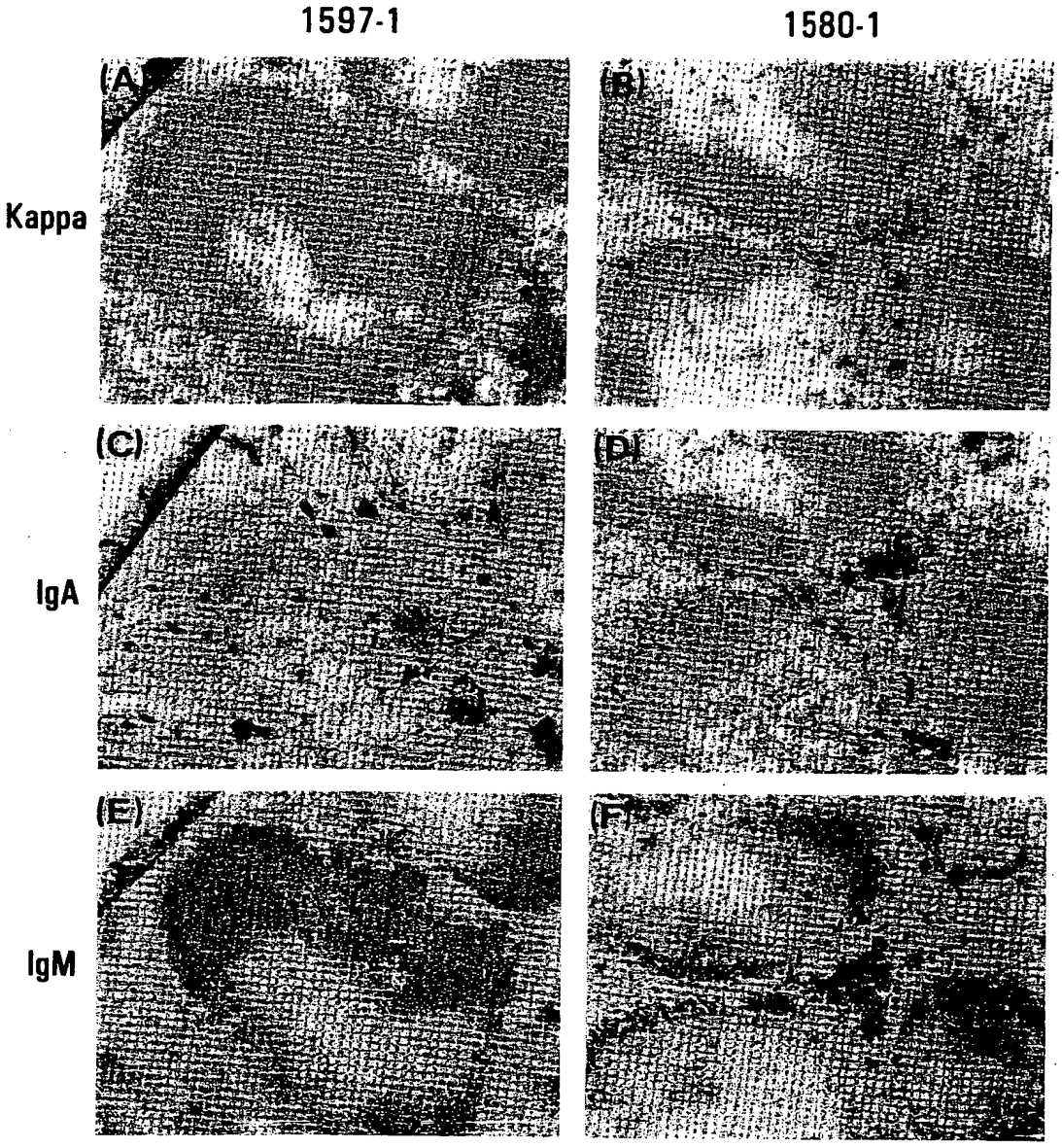

Figure 1. Cryostat sections of IgA transgenic mice. Serial sections of spleen from transgenic mice (line 1597-1: A, C, E; line 1580-1: B, D, F) were stained for κ (A, B), IgA (C, D), and IgM (E, F). Note that in both cases, cells staining positively for mouse IgA are restricted to B-dependent areas of the spleen white pulp, with isolated cells staining in the red pulp (presumably plasma cells). In line 1580-1 spleen. IgM^+ cells are present in only small numbers, but with no preferential localization within the B-dependent areas. In line 1597-1, IgA^+ cells are present in small numbers, but are more abundant than in control nontransgenic spleen, which stained positive for IgA only in rare scattered cells in the red pulp (not shown).

o-phenylenediamine dihydrochloride (Sigma, St. Louis, MO). Absorbances at a wavelength of 490 nm were determined on a Dynatech (Alexandria, VA) MR600 microplate reader.

3 Results

3.1 Generation of mouse α,κ transgenic mice

Twelve transgenic founder mice were generated and bred, and two of these lines (1580-1 and 1597-1) were selected for further study (Table 1). Mice from both lines showed expression of transgene mouse IgA evidenced by positive immunohistochemical staining of B cells in cryostat sections of spleen and lymph node (Fig. 1) and by serum levels of mouse IgA >800 μg/ml (*vs.* control levels <500 μg/ml).

Serum from control and transgenic 1580-1 mice were tested in ELISA for titers against PC using the mAb T15 as a standard. Total Ig (all classes) titers against PC were similar for all mice (range 8.8 to 13.3 μg/ml). In contrast, IgA titers against PC were less than 0.1 μg/ml in the control while two 1580-1 transgenic mice gave titers of 4.6 and 4.7 μg/ml. This indicated that while transgenic mice generated high titers of transgene IgA specific for PC, not all of the anti-PC activity was due to the transgene Ig. Considering that total IgA levels were much higher, these data also indicate that most (>97%) of the IgA (presumably transgene encoded) was not specific for PC.

On further study, line 1580-1 was found to have unusually low levels of endogenous IgM^+ cells (Fig. 2). In FCM analysis of spleen cells, IgM^+ cells comprised 10% of total κ^+ cells. Interestingly, transgenic spleen cells showed two distinct patterns of κ expression. The major group (Fig. 2c lower right quadrant) expressed low levels of κ, and co-expressed the IgA transgene. The minor group (Fig. 2c upper right quadrant) expressed κ levels equivalent to those of control spleen cells (Fig. 2a upper right quadrant), and co-expressed only endogenous IgM. By contrast, spleen cells from 1597-1 mice had normal numbers of IgM^+ cells; only a small group showed the low levels of κ expression, and these cells expressed IgM (Fig. 2e). Co-expression of transgene mouse IgA was not determined in these cells. Spleen cryostat sections of 1597-1 mice stained for mouse IgA revealed significant numbers of positive cells in B cell-dependent areas and red pulp (Fig. 1c). However, surface mouse IgA on 1597-1 spleen cells was almost as low as on nontransgenic spleen cells (Fig. 2f), suggesting that most transgene mouse IgA in 1597-1 mice is cytoplasmic.

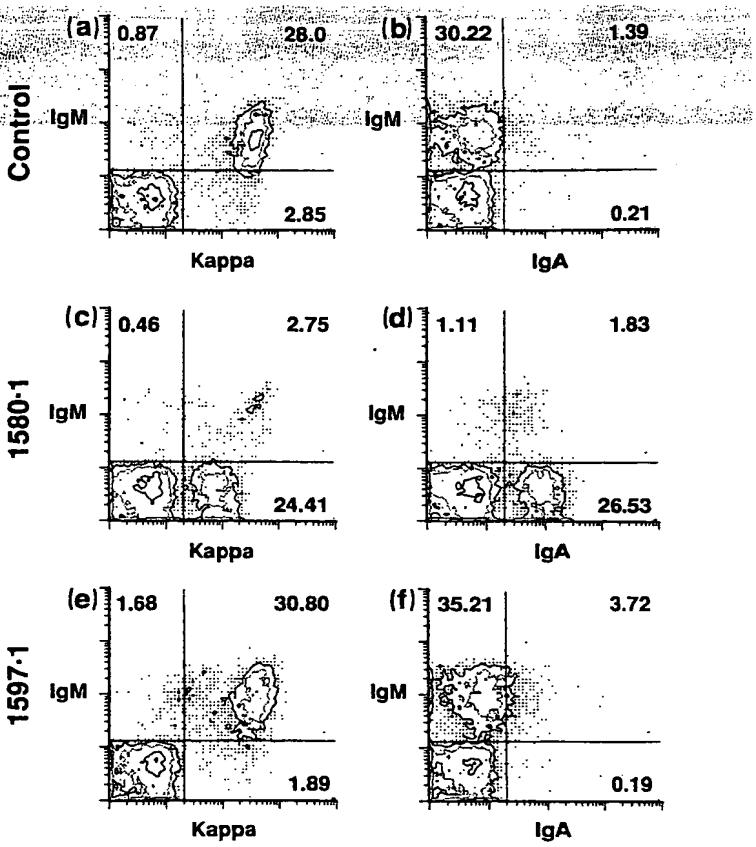

Figure 2. FCM on mouse lymphocytes from IgA transgenic mice. Spleen cell suspensions from control B6 (a, b), line 1580-1 (c, d) and line 1597-1 (e, f) mice were stained with combinations of FITC-anti-IgM/PE-anti-κ (a, c, e) and FITC-anti-IgM/PE-anti-IgA (b, d, f). Note that line 1580-1 spleen B cells are predominantly IgA^+/IgM^-.

Table 1. Transgenic mice sheep and pigs[a)]

Species	Founder no.	Transgenes integrated (No. of complete genes)	Mouse IgA/mouse κ expression
Mouse	1597-1	α (>10)/κ (>10)	Lymphocytes and serum IgA
	1580-1	α (2)/κ (2)	Lymphocytes and serum IgA
Pig	4203	α (2)	Lymphocytes and serum mouse IgA
	5001	α (6)/κ (1)	Lymphocytes and serum mouse IgA
Sheep	906	α (3)/κ (3)	Lymphocyte IgA only
	952	α (1)	No expression

a) In addition to the integrated intact transgenes listed above, mouse founder 1580-1 integrated two copies of a partial IgA fragment, and founder 1597 integrated two copies of a partial IgA gene fragment. Pig founder 4203 had two copies of a partial IgA transgene fragment, and founder 5001 integrated a single copy of a partial κ transgene, and six copies of a partial IgA fragment. Sheep founder 919 (not listed) integrated two copies of a partial κ fragment and two copies of a partial IgA fragment, animal 906 integrated three copies of a partial κ fragment, and animal 952 caried a partial IgA fragment.

In cryostat sections of 1580-1 lymphoid tissues, IgM^+ cells were scattered throughout the B-dependent areas (Fig. 1). The random distribution among κ^+ cells suggested that there were no functional differences between B cells expressing transgene IgA and those expressing endogenous Ig.

The total number of spleen cells in transgenic 1580-1 mice was also below normal levels, a phenomenon that was especially pronounced in younger mice. For example, in one pair of 4-week-old mice, the spleen from a control littermate contained 57×10^6 Ig^+ cells while the spleen from a 1580-1 transgenic mouse contained only 7×10^6 Ig^+ cells, an 8-fold reduction. Considering the drastically diminished percentage of IgM^+ cells and the fewer total spleen cells, total numbers of cells expressing endogenous Ig might be less than 200-fold below normal. It was therefore of concern whether adequate Ig responses could be generated in these animals. To address this issue, serum from control and 1580-1 transgenic mice was tested for total IgM using radial immunodiffusion. Our results indicated that while transgenic serum contained less IgM than controls (115 ± 48 μg/ml in four transgenic mice *vs.* 179 ± 16 μg/ml in thee age-matched controls, all 12 weeks of age), the levels were still near normal levels, and the range of values from transgenic and control mice overlapped.

3.2 Generation of mouse IgA transgenic pigs

Two live transgenic founder pigs were generated from microinjection of 542 embryos. Southern blot analysis indicated that only the mouse IgA transgene was integrated in both animals. Cytospin preparations of peripheral blood leukocytes were stained for mouse IgA and cells from both animals and their transgenic offspring demonstrated strong staining for the transgene mouse IgA protein (not shown). However, in both cases, the majority of Ig^+ B lymphocytes did not express the transgene. Thus, in line 4203 $<2\%$ of peripheral blood nucleated cells stained for mouse IgA while approximately 23%–45% of peripheral blood nucleated cells stained positive for pig Ig (6 animals tested). In line 5001, 8%–9% of nucleated cells expressed mouse IgA while 19%–20% of cells expressed pig Ig (2 animals tested). In transgenic offspring of both lines, lymph node and spleen cryostat sections stained positive for mouse IgA in B cell-dependent areas (Fig. 3).

Serum from these animals were also screened by radial immunodiffusion for secreted mouse IgA. While one founder (5001) was consistently negative up to 4 months of age, another founder (4203) demonstrated high levels of serum mouse IgA (300 μg/ml) starting at approximately 6 weeks of age. Transgenic offspring from both founders demonstrated high serum levels of mouse IgA as early as 5 weeks of age. Average levels were much higher in the offspring, up to 1380 μg/ml (range 390–1380, mean 630 ± 300, $n = 10$) for line 4203 offspring. In line 5001, three transgenic pigs at 35 days averaged 1293 μg/ml (range 1126–1421). Finally, the expression of the mouse transgene in pigs did not affect endogenous Ig expression; in pigs of both transgenic lines, serum levels of endogenous pig Ig were equivalent to those of nontransgenic littermates (approximately 20000 μg/ml in 19–22-week-old pigs, approximately 9000 μg/ml in 7-week pigs). Analysis of serum Ig levels over time (Fig. 4) indicated that the transgene mouse IgA production paralleled endogenous pig IgG production.

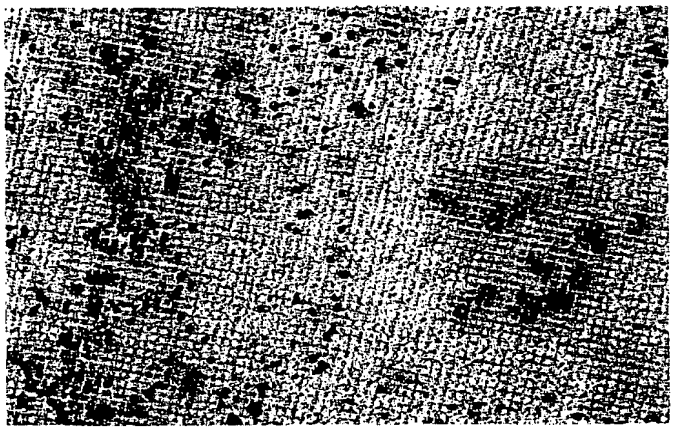

Figure 3. Cryostat sections of mouse IgA transgenic pig. A cryostat section from the spleen of a 22-week-old line 4203 transgenic pig was stained for mouse IgA. Strong staining was seen in cells in both white and red pulp. Tissue from nontransgenic littermates were negative for mouse IgA (not shown).

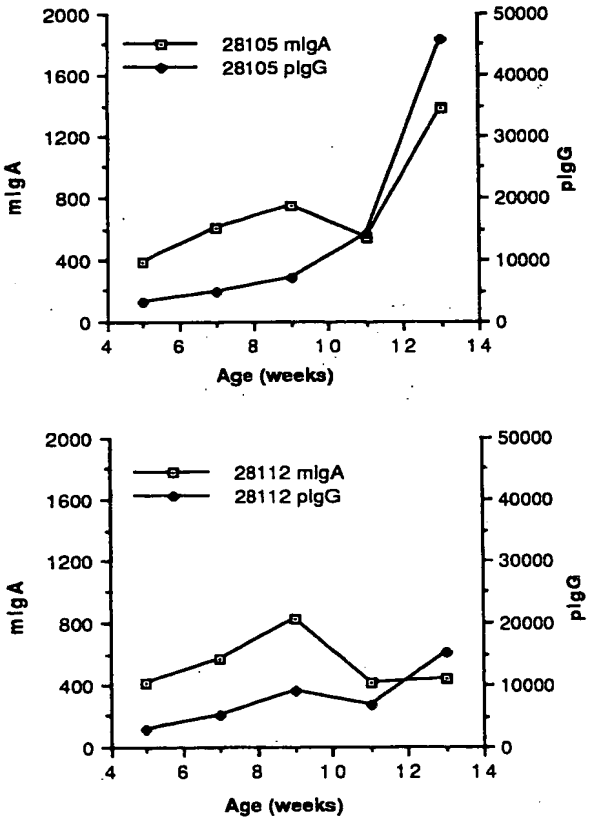

Figure 4. Serum Ig levels in transgenic pigs over time. Serum mouse IgA (mIgA) and pig IgG (pIgG) were quantitated on serial samples from two line 4203 transgenic pig littermates (numbered 29105 and 28112). Note that both transgenic mouse IgA and endogenous pig IgG increase with time in parallel fashion.

The serum from 4203 was further characterized by ELISA assays for mouse IgA titers against PC. Titers equivalent to $< 0.1\%$ of total serum mouse IgA were detected, suggesting that most ($> 99\%$) of the mouse IgA did not bind PC.

3.3 Generation of mouse IgA transgenic sheep

Three live founder transgenic sheep were generated from injection of 222 fertilized eggs, but of these, only two founders had intregrated intact transgenes. One founder (906) had integrated intact transgenes for both mouse IgA H chain and κ light chain, while the second (952) carried only a single α H chain transgene. Cytospin preparations of PBL from founder 906 stained positively for mouse IgA but not mouse κ, and serum tested negative for immunoreactive mouse IgA. In contrast, PBL and serum from sheep 952 were negative for mouse IgA.

4 Discussion

The results presented here show that mouse Ig transgenes can be expressed in lymphoid tissues in a variety of mammalian species. Previous concerns about transgene inhibition of endogenous Ig expression were partially justified as one transgenic mouse line showed severe inhibition of endogenous IgM expression and reduced numbers of B cells. However, this phenotype was the exception, for despite significant levels of expression of the mouse IgA in two lines of transgenic pigs, expression of endogenous pig Ig was not reduced, nor was the number of B cells in peripheral blood affected. In fact, in mouse line 1597-1, and both pig lines 4203 and 5001, the majority of B cells did not even express the transgene mouse IgA. These results support prospects for future "congenital immunization" in commercial animal species, and may also help in the development of models for gene therapy with eventual application to human disease.

In the transgenic mice, only a low proportion of serum transgene IgA was specific for PC. This may have a number of explanations. The anti-PC binding affinity of the transgene α/κ combination may be low, especially considering that the two genes were cloned from two different mAb (the κ from M167, and α from a prototypical T15). For example, in mice transgenic for the M167 κ gene, none of the anti-PC hybridomas isolated were found to use the prototypical T15 H chain sequence [8]. As a result, environmental antigen may drive maturation of transgenic α/endogenous L chain-expressing B cells more strongly than transgenic α/transgenic κ cells. Much of this transgenic α/endogenous L chain might in fact use the T15 κ, as a similar effect has been described before in Ig transgenic mice expressing one chain of the M167 antibody. So for example, transgenic mice bearing only a M167 IgM transgene expressed higher than normal levels of endogenous M167-like κ genes [5].

Finally, it is possible that the κ transgene was not expressed at all. Indeed, spleen cells from 1580-1 transgenic mice stained with an mAb specific for V167 κ were negative for the V167 κ determinant (J. Kenny, personal communication), suggesting that the L chain transgene was not expressed. A possible explanation is that the inability of the two transgene κ chains to form functional heterodimers may cause suppression of the κ transgene. Further studies with other Ig transgenes will be necessary to resolve this question.

In most cases, the expression of the transgene Ig genes had little or no effect on the secretion of endogenous Ig into the serum. This has been noted before in other transgenic mouse studies [5, 7], but the present study extends this observation to Ig transgenic pigs (and probably sheep). Subtle effects on the rearrangement and expression of endogenous Ig genes are more difficult to assess and will require further study to quantitate; however, our results suggest that the severe inhibition of endogenous IgM expression seen in mouse line 1580-1 is a relatively rare phenomenon. This phenotype was also observed in one line of IgG_{2b} transgenic mice while other IgG_{2b} lines showed little if any feedback inhibition (Lo, Storb et al., unpublished results).

It is not clear why some transgene integrants show the inhibition of endogenous IgM observed in 1580-1 mice. One possible explanation is that earlier expression of some transgene integrants in pre-B cells may have a more pronounced effect on endogenous genes. Alternatively, subtle differences in transgene number or site of integration may alter the splicing of the transcript and result in a greater proportion of membrane Ig. The membrane Ig may have strong negative feedback effects [5, 6]. In this context it is striking that the 1597-1 line has very low expression of surface mouse IgA, and no obvious effect on endogenous IgM expression.

Whatever the mechanism of feedback inhibitions, it appears to also have a profound effect on the overall numbes of B cells and T cells. It seems that transgenic Ig affects the overall proliferative capacity of the B cells. Indeed, as has been observed here in the pig transgenic lines, founder animals usually show far fewer transgene-positive cells in BM-derived cells (compared to transgenic offspring) as would be expected on the basis of its germ line or somatic tissue mosaicism. We have attributed this effect to the selective growth advantage of BM-derived cells that do not express the transgene.

In both pig transgenic lines, the mouse IgA was secreted into the serum despite the absence of any mouse L chain, so it was apparently able to form complexes with the endogenous pig L chain. While this affected the ability of the transgene to contribute to the production of high titers of specific antibody (in this case anti-PC), it suggests that the transgenic mouse IgA may be able to function as an antigen receptor in pig B cells. This is supported by the observation that levels of mouse IgA secretion paralleled secretion of endogenous pig Ig. Presumably, in combination with the diverse repertoire of pig L chains, the xenogeneic antibody molecules can bind to antigens, induce activation of specific B cells, and contribute to the antibody repertoire of the pigs. It will be of interest how such xenogeneic antibodies participate in normal pig immune responses, especially with respect to secretion into bile, saliva and milk.

Since xenogeneic H/L chain heterodimers were found in the serum of the transgenic pigs, it is not clear why they were not found in the single sheep founder (906). However,

it should be noted that the founder 5001 pig had no detectable serum mouse IgA, yet all transgenic offspring had high serum levels of mouse IgA. It is likely that bone marrow mosaicism in the founder sheep prevented transgenic B cells from forming a major component of the animal's immune system. Transgenic offspring of the founder 906 sheep may express mouse IgA in the serum. Alternatively, the mouse IgA transgenes may also have been disrupted on integration such that proper synthesis and secretion of the soluble form of mouse IgA was prevented.

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